Measurements of Protein Crystal Face Growth Rates

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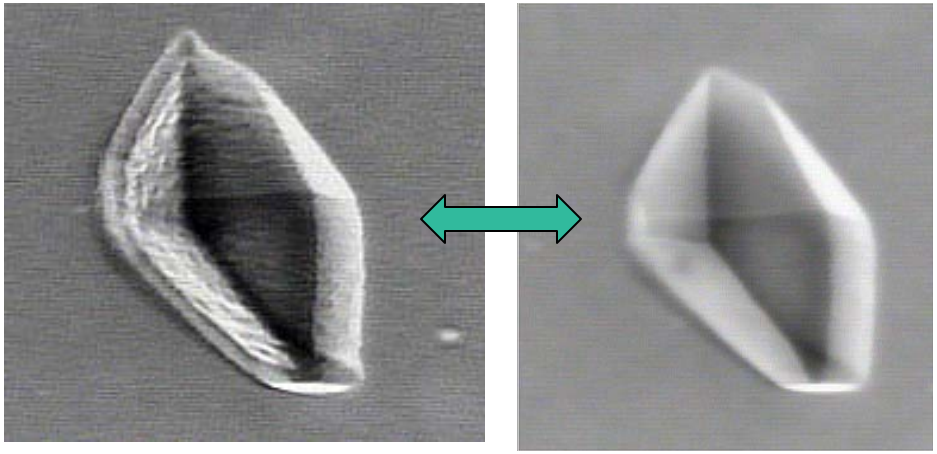
Overview

- Slow growth improves protein crystal X-ray diffraction quality (signal to noise ratio and resolution limit)
- Protein crystal quality can be enhanced by any number of means:
  1) Various additives or precipitating agents
  2) Varying supersaturation (density, temperature, etc.)
  3) Minimizing physical "handling"
  4) Chemical/genetic modification of proteins
  5) Microgravity
- NASA/CASIS have several flight projects that address the quality of protein crystals grown in μ-gravity environments.
- Ground based studies of physical processes that could determine crystal quality are also under investigation at MSFC.

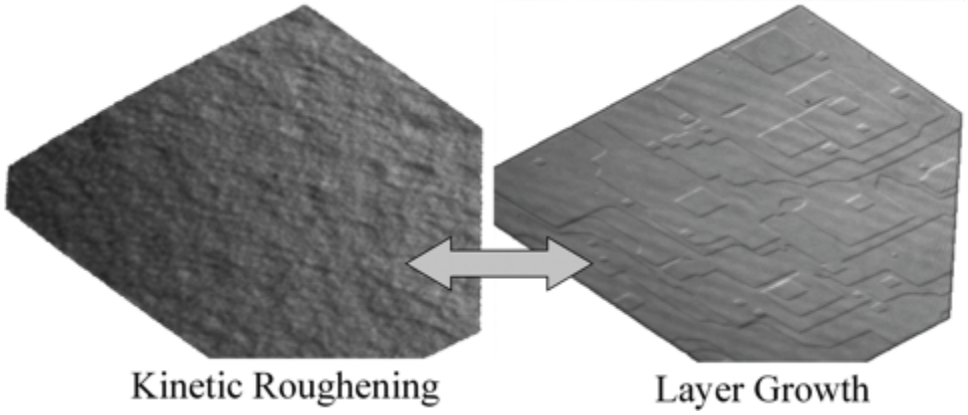
Kinetic Roughening



Kinetic Roughening of Lysozyme Crystals
S. Gorti, E.L. Forsythe & M.L. Pusey, Cryst. Growth & Design (2004) 4:691-699
S. Gorti, E.L. Forsythe & M.L. Pusey, Cryst. Growth & Design (2005) 5:473-482
S. Gorti, J. Konnert, E.L. Forsythe & M.L. Pusey, Cryst. Growth & Design (2005) 5:535-545

<u>Crossover Supersaturation</u>

Lysozyme: $\sigma = 2.0 \pm 0.2$
Glucose Isomerase: $\sigma = 5.0 \pm 0.1$



Kinetic Roughening of Glucose Isomerase Crystals
M. Sleutel, D. Maes, L. Wyns, and R. Willaert
Cryst. Growth Des., (2008) **8**:4409-4414

Note: A causal relationship between modes of crystal growth and X-ray diffraction "quality" has yet to be determined.

Crystal Quality

Journal of Crystal Growth 237–239 (2002) 295–299

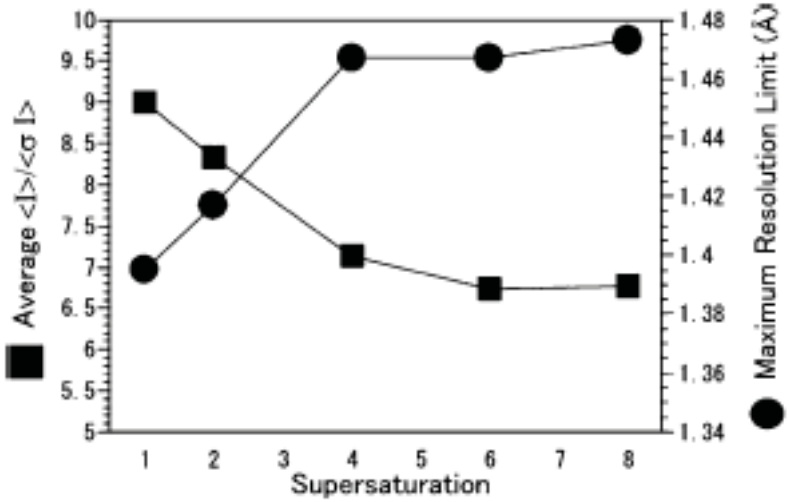


Fig. 1. Average maximum resolution limit (the line with circles) and average $\langle I \rangle / \langle \sigma I \rangle$ (the line with squares) of crystals from each supersaturation condition. Note that both the resolution limit and the average $\langle I \rangle / \langle \sigma I \rangle$ value are higher in lower supersaturated solution.

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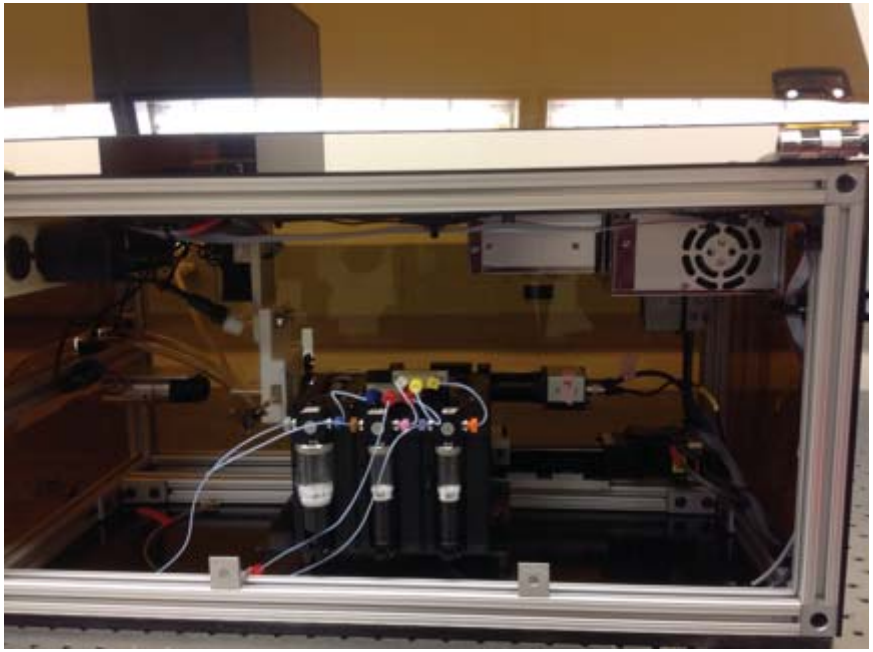
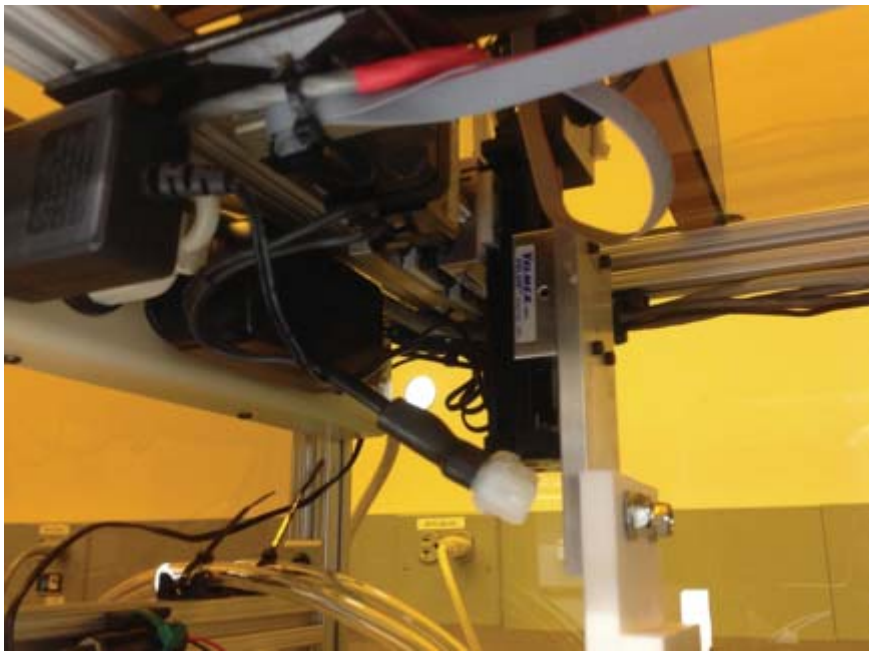
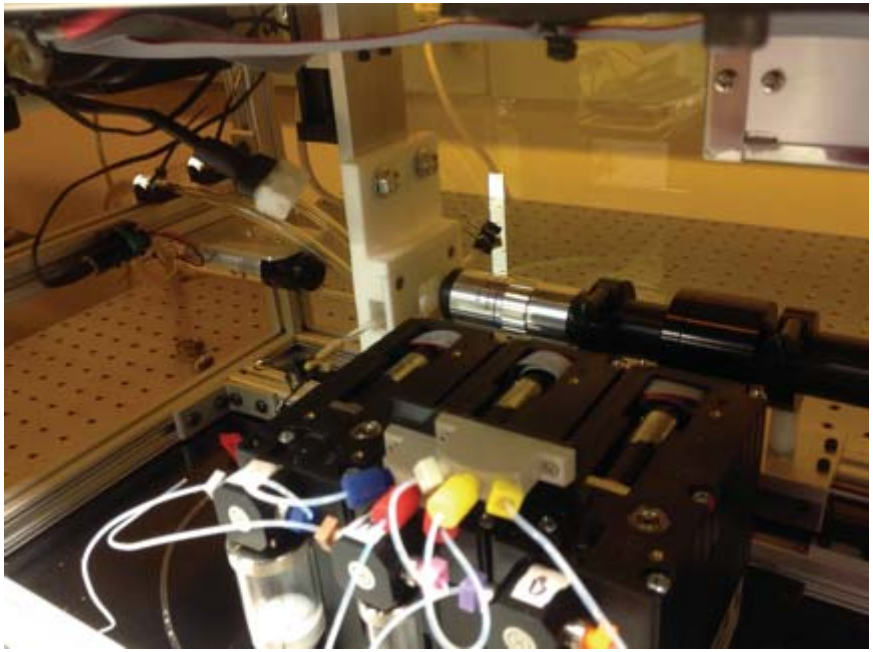
Face Growth Rate Apparatus

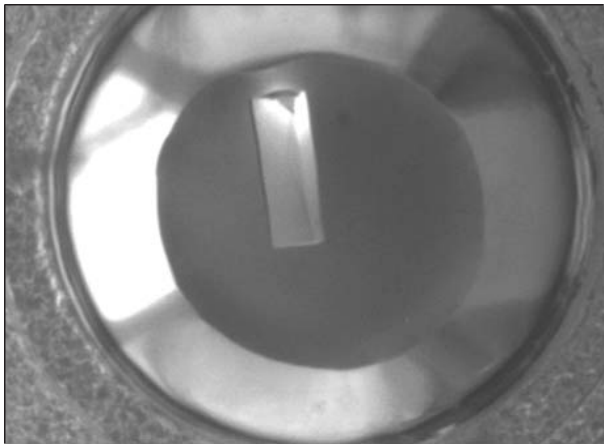


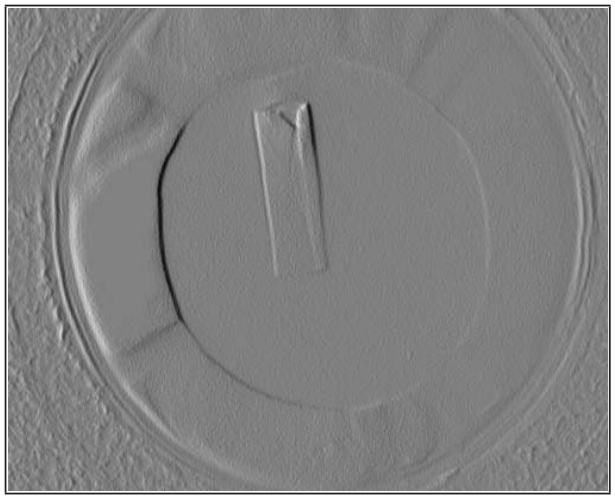
Closer Look at Translation Stages

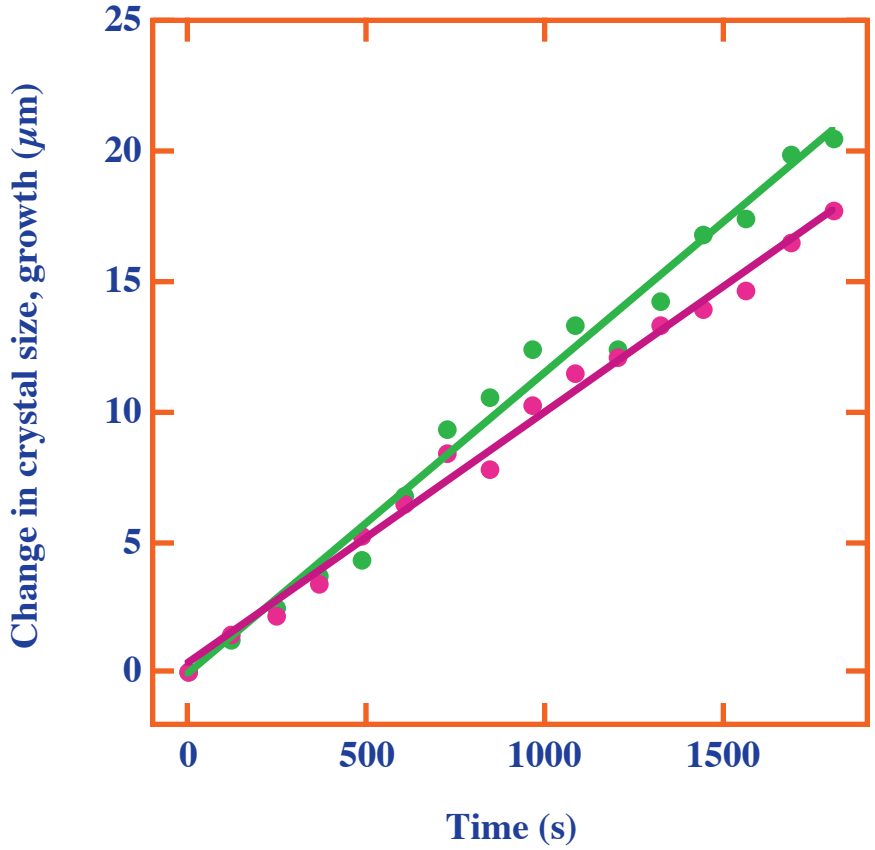




Measurement of Growth Rates

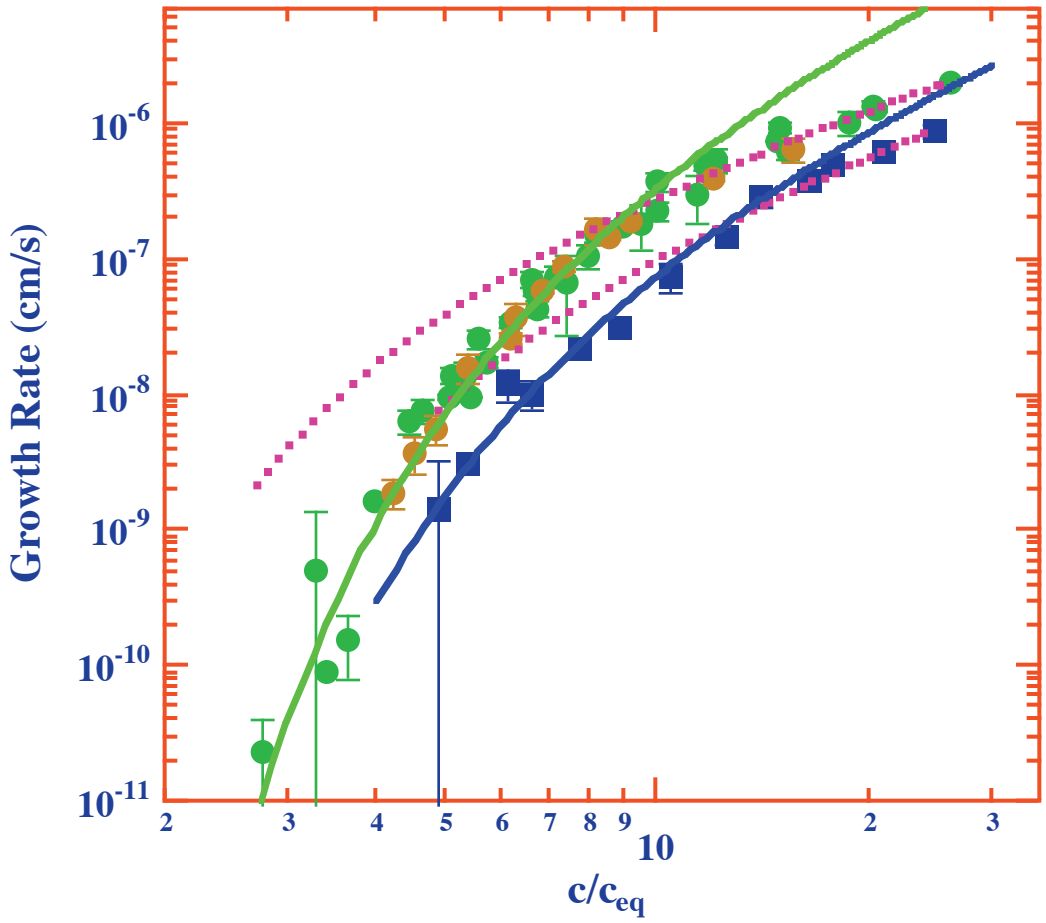






Average growth rate 1.1 ± 0.1 x 10^{-6} cm/s

Crystal Growth Rates



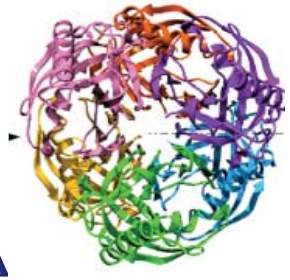
Protein Candidates

#1 – Inorganic PyroPhosphatase from *Thermococcus thioreducins*.

- Catalyzes cleavage of 1 pyrophosphate to 2x orthophosphate

  Found in all life

  Necessary for nucleic acid synthesis, any other reaction where a nucleotide triphosphate is converted to a nucleotide monophosphate + diphosphate.



- Hyperthermophile enzyme is active to 85°C
- Crystallizes very readily – current diffraction to ~1.1 Å
- Crystals currently grown on ISS for neutron diffraction studies.
- Very stable in X-ray beam at room temperature.

Protein Candidates (cont'd)

#2 Proliferating Cell Nuclear Antigen – (PCNA)

- A processivity factor that is essential for nucleic acid replication.
- Both hyperthermophile and psychophile versions available.
- Both forms are facile crystallizers.

#3 Glyoxylate reductase

- Catalyzes reduction of glyoxyate to glycolate.
- Crystallizes readily, attractive for neutron diffraction studies.

#4 Haloacid Dehalogenase

- Catalyzes conversion of a 2-haloacid to 2-hydroxyacid + halide.
- May be useful in chemical waste treatment.
- Nascent collaborative effort between iXG and another laboratory.

Summary

- Protein crystal growth rates will be determined for several hyperthermophile proteins.
- The growth rates will be assessed using available theoretical models, including kinetic roughening.
- If/when kinetic roughening supersaturations are established, determinations of protein crystal quality over a range of supersaturations will also be assessed.
- The results of our ground based effort may well address the existence of a correlation between fundamental growth mechanisms and protein crystal quality.